WEST

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Main Menu | Search Form | Posting Counts | Show S Numbers | Edit S Numbers | Preferences | Cases

Search Results -

Terms	Documents
L3 same (recombin$6 or clon$5 or isolat$5)	13

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Search: L4

Refine Search | Recall Text | Clear

Search History

DATE: Wednesday, June 04, 2003 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=OR			
L4	L3 same (recombin$6 or clon$5 or isolat$5)	13	L4
L3	L2 same carot$7	23	L3
L2	L1 same epsil$5	24	L2
L1	lycop$5 same cyclas$5	92	L1

END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 10 of 13 returned.

☐ 1. Document ID: US 20030003528 A1

L4: Entry 1 of 13 File: PGPB Jan 2, 2003

PGPUB-DOCUMENT-NUMBER: 20030003528
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030003528 A1

TITLE: Carotenoid production from a single carbon substrate

PUBLICATION-DATE: January 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Brzostowicz, Patricia C.	West Chester	PA	US	
Cheng, Qiong	Wilmington	DE	US	
Dicosimo, Deana	Rockland	DE	US	
Koffas, Mattheos	Wilmington	DE	US	
Miller, Edward S.	Wilmington	DE	US	
Odom, James M.	Kennett Square	PA	US	
Picataggio, Stephen K.	Landenberg	PA	US	
Rouviere, Pierre E.	Wilmington	DE	US	

US-CL-CURRENT: 435/67; 435/252.3

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 20020177181 A1

L4: Entry 2 of 13 File: PGPB Nov 28, 2002

PGPUB-DOCUMENT-NUMBER: 20020177181
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020177181 A1

TITLE: Increasing bioavailability of carotenoids

PUBLICATION-DATE: November 28, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Kanner, Joseph	Rehovot		IL	
Levy, Arieh	Rehovot		IL	
Granit, Rina	Rehovot		IL	

US-CL-CURRENT: 435/19; 435/67

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWMC | Draw Desc | Image

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☐ 3. Document ID: US 20020086380 A1

L4: Entry 3 of 13 File: PGPB Jul 4, 2002

PGPUB-DOCUMENT-NUMBER: 20020086380
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020086380 A1

TITLE: GENES ENCODING EPSILON LYCOPENE CYCLASE AND METHOD FOR PRODUCING BICYCLIC CAROTENE

PUBLICATION-DATE: July 4, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
CUNNINGHAM JR, FRANCIS X.	CHEVY CHASE	MD	US	

US-CL-CURRENT: 435/183; 435/232, 435/252.3, 435/320.1, 435/410, 435/411, 514/763, 585/23

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWMC | Draw Desc | Image

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☐ 4. Document ID: US 20020053096 A1

L4: Entry 4 of 13 File: PGPB May 2, 2002

PGPUB-DOCUMENT-NUMBER: 20020053096
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020053096 A1

TITLE: Polynucleotide molecule from Haematococcus pluvialis encoding a polypeptide having a beta-C-4-oxygenase activity for biotechnological production of (3S,3'S) astaxanthin and its specific expression in chromoplasts of higher plants

PUBLICATION-DATE: May 2, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Hirschberg, Joseph	Jerusalem		IL	
Lotan, Tamar	Kineret		IL	

US-CL-CURRENT: 800/282; 435/252.3, 435/320.1, 435/67, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWMC | Draw Desc | Image

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☐ 5. Document ID: US 20020051998 A1

L4: Entry 5 of 13 File: PGPB May 2, 2002

PGPUB-DOCUMENT-NUMBER: 20020051998
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020051998 A1

TITLE: Directed evolution of biosynthetic and biodegradation pathways

PUBLICATION-DATE: May 2, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Schmidt-Dannert, Claudia	Shoreview	MN	US	
Arnold, Frances H.	Pasadena	CA	US	

US-CL-CURRENT: 435/7.1; 435/325, 435/410, 435/67

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 6. Document ID: US 6524811 B1

L4: Entry 6 of 13 File: USPT Feb 25, 2003

US-PAT-NO: 6524811
DOCUMENT-IDENTIFIER: US 6524811 B1

TITLE: Methods of increasing or decreasing carotenoids and other isoprenoids using IPP isomerase

DATE-ISSUED: February 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cunningham, Jr.; Francis X.	Chevy Chase	MD		
Sun; Zairen	Hyattsville	MD		

US-CL-CURRENT: 435/67; 435/233

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 7. Document ID: US 6232530 B1

L4: Entry 7 of 13 File: USPT May 15, 2001

US-PAT-NO: 6232530
DOCUMENT-IDENTIFIER: US 6232530 B1
**** See image for Certificate of Correction ****

TITLE: Marigold DNA encoding beta-cyclase

DATE-ISSUED: May 15, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
DellaPenna; Dean	Reno	NV		
Cunningham, Jr.; Francis X.	Chevy Chase	MD		

US-CL-CURRENT: 800/282; 536/23.2, 536/23.6

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 8. Document ID: US 6218599 B1

L4: Entry 8 of 13 File: USPT Apr 17, 2001

US-PAT-NO: 6218599
DOCUMENT-IDENTIFIER: US 6218599 B1

TITLE: Polynucleotide molecule from Haematococcus pluvialis encoding a polypeptide having a .beta.-C-4-oxygenase activity for biotechnological production of (3S, 3'S) astaxanthin and its specific expression in chromoplasts of higher plants

DATE-ISSUED: April 17, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hirschberg; Joseph	Jerusalem			IL
Lotan; Tamar	Kineret			IL

US-CL-CURRENT: 800/295; 435/189, 435/252.3, 435/252.33, 435/254.11, 435/320.1, 435/410, 536/23.1, 536/23.2, 536/23.74, 800/298

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 9. Document ID: US 5965795 A

L4: Entry 9 of 13 File: USPT Oct 12, 1999

US-PAT-NO: 5965795
DOCUMENT-IDENTIFIER: US 5965795 A

TITLE: Polynucleotide molecule from Haematococcus pluvialis encoding a polypeptide having a beta-C-4-oxygenase activity for biotechnological production of (3S, 3'S) astaxanthin and its specific expression in chromoplasts of higher plants

DATE-ISSUED: October 12, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hirschberg; Joseph	Jerusalem			IL
Lotan; Tamar	Kineret			IL

US-CL-CURRENT: 800/295; 435/183, 435/189, 435/252.3, 435/252.33, 435/254.11, 435/254.21, 435/320.1, 435/410, 536/23.1, 536/23.2, 536/23.74

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Image | KWMC | Draw Desc

☐ 10. Document ID: US 5935808 A

L4: Entry 10 of 13 File: USPT Aug 10, 1999

US-PAT-NO: 5935808
DOCUMENT-IDENTIFIER: US 5935808 A

TITLE: Carotenoid-producing bacterial species and process for production of carotenoids using same

DATE-ISSUED: August 10, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hirschberg; Joseph	Jerusalem			IL
Harker; Mark	Jerusalem			IL

US-CL-CURRENT: 435/67; 435/252.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Image | KWMC | Draw Desc

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Display Format: - Change Format

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Search Results - Record(s) 11 through 13 of 13 returned.

☐ 11. Document ID: US 5744341 A

L4: Entry 11 of 13 File: USPT Apr 28, 1998

US-PAT-NO: 5744341
DOCUMENT-IDENTIFIER: US 5744341 A

TITLE: Genes of carotenoid biosynthesis and metabolism and a system for screening for such genes

DATE-ISSUED: April 28, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cunningham, Jr.; Francis X.	Chevy Chase	MD		
Sun; Zairen	Hyattsville	MD		

US-CL-CURRENT: 435/189; 435/252.3, 435/254.11, 435/320.1, 435/325, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 12. Document ID: WO 9961399 A1 AU 9943084 A BR 9911597 A EP 1080057 A1 JP 2002516077 W US 20020086380 A1

L4: Entry 12 of 13 File: DWPI Dec 2, 1999

DERWENT-ACC-NO: 2000-062667
DERWENT-WEEK: 200323

TITLE: New eukaryotic epsilon-lycopene cyclase and related DNA, used to regulate carotenoid synthesis in plants and other hosts, e.g. for treatment of cancer

INVENTOR: CUNNINGHAM, F X

PRIORITY-DATA: 1998US-0084222 (May 26, 1998), 1996US-0624125 (March 29, 1996), 1997US-0937155 (September 25, 1997)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 9961399 A1	December 2, 1999	E	037	C07C013/00
AU 9943084 A	December 13, 1999		000	
BR 9911597 A	February 13, 2001		000	C07C013/00
EP 1080057 A1	March 7, 2001	E	000	C07C013/00
JP 2002516077 W	June 4, 2002		042	C12N015/09
US 20020086380 A1	July 4, 2002		000	C12N009/00

INT-CL (IPC): A01 N 27/00; A61 K 31/045; A61 P 35/00; A61 P 43/00; C07 C 13/00; C12 N 1/21; C12 N 5/00; C12 N 5/10; C12 N 9/00; C12 N 9/90; C12 N 15/00; C12 N 15/09; C12 N 1/21; C12 R 1:19

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWMC | Draw. Desc | Image

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☐ 13. Document ID: WO 9955887 A2 AU 9937491 A EP 1071800 A2

L4: Entry 13 of 13 File: DWPI Nov 4, 1999

DERWENT-ACC-NO: 2000-062037
DERWENT-WEEK: 200005

TITLE: Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme

INVENTOR: CAHOON, R E; KINNEY, A J ; PEARLSTEIN, R W ; WILLIAMS, M E

PRIORITY-DATA: 1998US-083042P (April 24, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 9955887 A2	November 4, 1999	E	061	C12N015/82
AU 9937491 A	November 16, 1999		000	
EP 1071800 A2	January 31, 2001	E	000	C12N015/82

INT-CL (IPC): A01 H 5/00; C12 N 5/10; C12 N 9/00; C12 N 9/02; C12 N 15/53; C12 N 15/82; G01 N 33/50

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWMC | Draw. Desc | Image

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SEA LYCOPE? AND CYCLAS?

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L1 QUE LYCOPE? AND CYCLAS?

FILE 'DGENE, CAPLUS, USPATFULL, GENBANK, BIOSIS, SCISEARCH, MEDLINE, BIOTECHNO, ESBIOBASE, CABA, EMBASE, LIFESCI, BIOTECHDS, WPIDS, AGRICOLA, PASCAL, FROSTI, TOXCENTER, IFIPAT' ENTERED AT 17:11:30 ON 04 JUN 2003

L2 1275 S LYCOPE? (S) CYCLAS?
L3 982 S L2 (S) CAROTE?
L4 298 S L3 (S) EPSIL?
L5 216 DUP REM L4 (82 DUPLICATES REMOVED)
L6 4 S L5 (S) ADON?
L7 2 S L5 (S) PALAES?
L8 63 S L5 (S) (RECOMBIN? OR CLON? OR ISOL?)

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NEWS 3	Jun 03	New e-mail delivery for search results now available
NEWS 4	Aug 08	PHARMAMarketLetter(PHARMAML) - new on STN
NEWS 5	Aug 19	Aquatic Toxicity Information Retrieval (AQUIRE) now available on STN
NEWS 6	Aug 26	Sequence searching in REGISTRY enhanced
NEWS 7	Sep 03	JAPIO has been reloaded and enhanced
NEWS 8	Sep 16	Experimental properties added to the REGISTRY file
NEWS 9	Sep 16	CA Section Thesaurus available in CAPLUS and CA
NEWS 10	Oct 01	CASREACT Enriched with Reactions from 1907 to 1985
NEWS 11	Oct 24	BEILSTEIN adds new search fields
NEWS 12	Oct 24	Nutraceuticals International (NUTRACEUT) now available on STN
NEWS 13	Nov 18	DKILIT has been renamed APOLLIT
NEWS 14	Nov 25	More calculated properties added to REGISTRY
NEWS 15	Dec 04	CSA files on STN
NEWS 16	Dec 17	PCTFULL now covers WP/PCT Applications from 1978 to date
NEWS 17	Dec 17	TOXCENTER enhanced with additional content
NEWS 18	Dec 17	Adis Clinical Trials Insight now available on STN
NEWS 19	Jan 29	Simultaneous left and right truncation added to COMPENDEX, ENERGY, INSPEC
NEWS 20	Feb 13	CANCERLIT is no longer being updated
NEWS 21	Feb 24	METADEX enhancements
NEWS 22	Feb 24	PCTGEN now available on STN
NEWS 23	Feb 24	TEMA now available on STN
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NEWS 25	Feb 26	PCTFULL now contains images
NEWS 26	Mar 04	SDI PACKAGE for monthly delivery of multifile SDI results
NEWS 27	Mar 20	EVENTLINE will be removed from STN
NEWS 28	Mar 24	PATDPAFULL now available on STN
NEWS 29	Mar 24	Additional information for trade-named substances without structures available in REGISTRY
NEWS 30	Apr 11	Display formats in DGENE enhanced
NEWS 31	Apr 14	MEDLINE Reload
NEWS 32	Apr 17	Polymer searching in REGISTRY enhanced
NEWS 33	Apr 21	Indexing from 1947 to 1956 being added to records in CA/CAPLUS
NEWS 34	Apr 21	New current-awareness alert (SDI) frequency in WPIDS/WPINDEX/WPIX
NEWS 35	Apr 28	RDISCLOSURE now available on STN
NEWS 36	May 05	Pharmacokinetic information and systematic chemical names added to PHAR
NEWS 37	May 15	MEDLINE file segment of TOXCENTER reloaded
NEWS 38	May 15	Supporter information for ENCOMPPAT and ENCOMPLIT updated
NEWS 39	May 16	CHEMREACT will be removed from STN
NEWS 40	May 19	Simultaneous left and right truncation added to WSCA
NEWS 41	May 19	RAPRA enhanced with new search field, simultaneous left and right truncation

NEWS EXPRESS	April 4 CURRENT WINDOWS VERSION IS V6.01a, CURRENT MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP), AND CURRENT DISCOVER FILE IS DATED 01 APRIL 2003
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=> index bioscience medicine
FILE 'DRUGMONOG' ACCESS NOT AUTHORIZED
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FULL ESTIMATED COST                                     0.21       0.21

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       CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE,
       DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 17:09:01 ON 04 JUN 2003

70 FILES IN THE FILE LIST IN STNINDEX

Enter SET DETAIL ON to see search term postings or to view
search error messages that display as 0* with SET DETAIL OFF.

=> s lycope? and cyclas?
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          2   FILE ANABSTR
          5   FILE AQUASCI
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          2   FILE BIOCOMMERCE
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         38   FILE BIOTECHABS
         38   FILE BIOTECHDS
         49   FILE BIOTECHNO
         46   FILE CABA
          2   FILE CANCERLIT
        156   FILE CAPLUS
          6   FILE CEABA-VTB
          1   FILE CONFSCI
          2   FILE CROPB
          8   FILE CROPU
        343   FILE DGENE
          1   FILE DRUGU
          1   FILE EMBAL
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         49   FILE ESBIOBASE
          1   FILE FEDRIP
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         21   FILE IFIPAT
          9   FILE JICST-EPLUS
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         68   FILE MEDLINE
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          1   FILE NTIS
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L1    QUE LYCOPE? AND CYCLAS?

=> d rank
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F2          156   CAPLUS
F3          112   USPATFULL
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F30           2   BIOCOMMERCE
F31           2   CANCERLIT
F32           2   CROPB
F33           1   CONFSCI
F34           1   DRUGU
F35           1   EMBAL
F36           1   FEDRIP
F37           1   NTIS
F38           1   OCEAN


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                                               ENTRY     SESSION
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=> s lycope? (s) cyclas?
L2 1275 LYCOPE? (S) CYCLAS?

=> s l2 (s) carote?
L3 982 L2 (S) CAROTE?

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=> s l3 (s) epsil?
L4          298 L3 (S) EPSIL?

=> dup rem l4
DUPLICATE IS NOT AVAILABLE IN 'DGENE, GENBANK'.
ANSWERS FROM THESE FILES WILL BE CONSIDERED UNIQUE
PROCESSING COMPLETED FOR L4
L5          216 DUP REM L4 (82 DUPLICATES REMOVED)

=> s l5 (s) adon?
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L75 (S) ADON?'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L77 (S) ADON?'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L79 (S) ADON?'
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L6            4 L5 (S) ADON?

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L7            2 L5 (S) PALAES?

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     (FILE 'HOME' ENTERED AT 17:08:45 ON 04 JUN 2003)

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     CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,
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                SEA LYCOPE? AND CYCLAS?
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                5   FILE AQUASCI
                6   FILE BIOBUSINESS
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               46   FILE CABA
                2   FILE CANCERLIT
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                1   FILE CONFSCI
                2   FILE CROPB
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              343   FILE DGENE
                1   FILE DRUGU
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               49   FILE ESBIOBASE
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              112   FILE USPATFULL
                3   FILE USPAT2
               29   FILE WPIDS
               29   FILE WPINDEX
L1               QUE LYCOPE? AND CYCLAS?
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     FILE 'DGENE, CAPLUS, USPATFULL, GENBANK, BIOSIS, SCISEARCH, MEDLINE,
     BIOTECHNO, ESBIOBASE, CABA, EMBASE, LIFESCI, BIOTECHDS, WPIDS, AGRICOLA,
     PASCAL, FROSTI, TOXCENTER, IFIPAT' ENTERED AT 17:11:30 ON 04 JUN 2003
L2          1275 S LYCOPE? (S) CYCLAS?
L3           982 S L2 (S) CAROTE?
L4           298 S L3 (S) EPSIL?
L5           216 DUP REM L4 (82 DUPLICATES REMOVED)
L6             4 S L5 (S) ADON?
L7             2 S L5 (S) PALAES?

=> d ti l6 1-4

L6    ANSWER 1 OF 4  DGENE (C) 2003 THOMSON DERWENT
TI    Identifying enzyme-catalyzing domain in carotenoid-synthesizing enzyme,
      by chimeric polynucleotide encoding chimeric carotenoid-synthesizing
      enzyme, truncation of enzymes or by site-directed mutation of enzymes -

L6    ANSWER 2 OF 4  DGENE (C) 2003 THOMSON DERWENT
TI    New eukaryotic epsilon-lycopene cyclase and related DNA, used to regulate
      carotenoid synthesis in plants and other hosts, e.g. for treatment of
      cancer  -

L6    ANSWER 3 OF 4  DGENE (C) 2003 THOMSON DERWENT
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TI New eukaryotic epsilon-lycopene cyclase and related DNA, used to regulate carotenoid synthesis in plants and other hosts, e.g. for treatment of cancer -

L6 ANSWER 4 OF 4 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Producing carotenoid compounds e.g. antheraxanthin and astaxanthin, by using microorganisms having a nucleic acid molecule encoding enzymes in the carotenoid biosynthetic pathway and which metabolize single carbon substrates;
carotenoid production via bacterium, fungus host cell transformation using enzyme for the steroid production, flavor and electro-optic compound

=> d ti l7 1-2

L7 ANSWER 1 OF 2 DGENE (C) 2003 THOMSON DERWENT
TI New eukaryotic epsilon-lycopene cyclase and related DNA, used to regulate carotenoid synthesis in plants and other hosts, e.g. for treatment of cancer -

L7 ANSWER 2 OF 2 DGENE (C) 2003 THOMSON DERWENT
TI New eukaryotic epsilon-lycopene cyclase and related DNA, used to regulate carotenoid synthesis in plants and other hosts, e.g. for treatment of cancer -

=> s l5 (s) (recombin? or clon? or isol?)
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PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L177 (S) '
L8 63 L5 (S) (RECOMBIN? OR CLON? OR ISOL?)

=> d ti l8 1-63

L8 ANSWER 1 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 2 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 3 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides

used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 4 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 5 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 6 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 7 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 8 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 9 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 10 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 11 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 12 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 13 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI New eukaryotic epsilon-lycopene cyclase and related DNA, used to regulate carotenoid synthesis in plants and other hosts, e.g. for treatment of cancer -

L8 ANSWER 14 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI New eukaryotic epsilon-lycopene cyclase and related DNA, used to regulate carotenoid synthesis in plants and other hosts, e.g. for treatment of cancer -

L8 ANSWER 15 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel method for regulating carotenoid biosynthesis in Marigolds by modulating the catalytic activity of beta- cyclase, beta-hydroxylase, epsilon cyclase, or IPP isomerase -

L8 ANSWER 16 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel method for regulating carotenoid biosynthesis in Marigolds by modulating the catalytic activity of beta- cyclase, beta-hydroxylase, epsilon cyclase, or IPP isomerase -

L8 ANSWER 17 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel method for regulating carotenoid biosynthesis in Marigolds by modulating the catalytic activity of beta- cyclase, beta-hydroxylase, epsilon cyclase, or IPP isomerase -

L8 ANSWER 18 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel method for regulating carotenoid biosynthesis in Marigolds by

modulating the catalytic activity of beta- cyclase, beta-hydroxylase, epsilon cyclase, or IPP isomerase -

L8 ANSWER 19 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel isolated lycopene epsilon cyclase polypeptide, useful for producing lutein, feed supplement and enhanced food products -

L8 ANSWER 20 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel isolated lycopene epsilon cyclase polypeptide, useful for producing lutein, feed supplement and enhanced food products -

L8 ANSWER 21 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel isolated lycopene epsilon cyclase polypeptide, useful for producing lutein, feed supplement and enhanced food products -

L8 ANSWER 22 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel isolated lycopene epsilon cyclase polypeptide, useful for producing lutein, feed supplement and enhanced food products -

L8 ANSWER 23 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 24 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 25 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 26 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 27 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 28 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 29 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 30 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel carotenoid biosynthesis enzyme polynucleotides and polypeptides used to identify inhibitors and to modulate expression of the enzyme -

L8 ANSWER 31 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI New eukaryotic epsilon-lycopene cyclase and related DNA, used to regulate carotenoid synthesis in plants and other hosts, e.g. for treatment of cancer -

L8 ANSWER 32 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI New eukaryotic epsilon-lycopene cyclase and related DNA, used to regulate carotenoid synthesis in plants and other hosts, e.g. for treatment of cancer -

L8 ANSWER 33 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel method for regulating carotenoid biosynthesis in Marigolds by modulating the catalytic activity of beta- cyclase, beta-hydroxylase, epsilon cyclase, or IPP isomerase -

L8 ANSWER 34 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel method for regulating carotenoid biosynthesis in Marigolds by modulating the catalytic activity of beta- cyclase, beta-hydroxylase, epsilon cyclase, or IPP isomerase -

L8 ANSWER 35 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel method for regulating carotenoid biosynthesis in Marigolds by modulating the catalytic activity of beta- cyclase, beta-hydroxylase, epsilon cyclase, or IPP isomerase -

L8 ANSWER 36 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Novel method for regulating carotenoid biosynthesis in Marigolds by modulating the catalytic activity of beta- cyclase, beta-hydroxylase, epsilon cyclase, or IPP isomerase -

L8 ANSWER 37 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Altering xanthophyll content of seeds by transformation - used to produce seed oils of increased carotenoid content, e.g. Brassica and cotton

L8 ANSWER 38 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Altering xanthophyll content of seeds by transformation - used to produce seed oils of increased carotenoid content, e.g. Brassica and cotton

L8 ANSWER 39 OF 63 DGENE (C) 2003 THOMSON DERWENT
TI Use of constructs comprising a carotenoid biosynthesis gene - for producing plants and seeds having altered carotenoid levels, modified fatty acid compositions or altered tocopherol levels.

L8 ANSWER 40 OF 63 CAPLUS COPYRIGHT 2003 ACS
TI cDNAs for the synthesis of cyclic carotenoids in petals of Gentiana lutea and their regulation during flower development

L8 ANSWER 41 OF 63 CAPLUS COPYRIGHT 2003 ACS
TI **Cloning** and sequencing of **lycopene .epsilon. cyclase** from romaine lettuce and use of the **cyclase** for producing bicyclic **carotene** and for treating disease

L8 ANSWER 42 OF 63 CAPLUS COPYRIGHT 2003 ACS
TI Cloning and sequencing of lycopene .epsilon. cyclase from spinach and production of lutein in microorganisms by expression of the lycopene .epsilon. cyclase

L8 ANSWER 43 OF 63 CAPLUS COPYRIGHT 2003 ACS
TI One ring or two? determination of ring number in carotenoids by lycopene .epsilon.-cyclases

L8 ANSWER 44 OF 63 CAPLUS COPYRIGHT 2003 ACS
TI Genes for enzymes of carotenoid biosynthesis and metabolism of bacteria and plants and their uses

L8 ANSWER 45 OF 63 CAPLUS COPYRIGHT 2003 ACS
TI Genes encoding epsilon lycopene cyclase and method for producing bicyclicepsilon carotene

L8 ANSWER 46 OF 63 CAPLUS COPYRIGHT 2003 ACS
TI Plant lycopene .epsilon.-cyclase and .beta.-carotene hydroxylase and lycopene cyclase enzymes and their encoding cDNAs

L8 ANSWER 47 OF 63 CAPLUS COPYRIGHT 2003 ACS
TI Regulation of carotenoid biosynthesis during tomato fruit development: expression of the gene for lycopene epsilon-cyclase is down-regulated during ripening and is elevated in the mutant Delta

L8 ANSWER 48 OF 63 USPATFULL
TI Methods of increasing or decreasing carotenoids and other isoprenoids using IPP isomerase

L8 ANSWER 49 OF 63 USPATFULL
TI Carotenoid production from a single carbon substrate

L8 ANSWER 50 OF 63 USPATFULL
TI Increasing bioavailability of carotenoids

L8 ANSWER 51 OF 63 USPATFULL
TI Polynucleotide molecule from Haematococcus pluvialis encoding a polypeptide having a beta-C-4-oxygenase activity for biotechnological production of (3S,3'S) astaxanthin and its specific expression in chromoplasts of higher plants

L8 ANSWER 52 OF 63 USPATFULL
TI Directed evolution of biosynthetic and biodegradation pathways

L8 ANSWER 53 OF 63 USPATFULL
TI Marigold DNA encoding beta-cyclase

L8 ANSWER 54 OF 63 USPATFULL
TI Polynucleotide molecule from Haematococcus pluvialis encoding a polypeptide having a .beta.-C-4-oxygenase activity for biotechnological production of (3S, 3'S) astaxanthin and its specific expression in chromoplasts of higher plants

L8 ANSWER 55 OF 63 USPATFULL
TI Polynucleotide molecule from Haematococcus pluvialis encoding a polypeptide having a beta-C-4-oxygenase activity for biotechnological production of (3S, 3'S) astaxanthin and its specific expression in chromoplasts of higher plants

L8 ANSWER 56 OF 63 USPATFULL
TI Carotenoid-producing bacterial species and process for production of carotenoids using same

L8 ANSWER 57 OF 63 USPATFULL
TI Genes of carotenoid biosynthesis and metabolism and a system for screening for such genes

L8 ANSWER 58 OF 63 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Expression and functional analysis of a gene cluster involved in the synthesis of decaprenoxanthin reveals the mechanisms for C50 carotenoid formation.

L8 ANSWER 59 OF 63 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI cDNAs for the synthesis of cyclic carotenoids in petals of Gentiana lutea and their regulation during flower development

L8 ANSWER 60 OF 63 CABA COPYRIGHT 2003 CABI
TI Xanthophylls and excess-energy dissipation: a genetic dissection in Arabidopsis.

L8 ANSWER 61 OF 63 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Producing carotenoid compounds e.g. antheraxanthin and astaxanthin, by using microorganisms having a nucleic acid molecule encoding enzymes in the carotenoid biosynthetic pathway and which metabolize single carbon substrates;
carotenoid production via bacterium, fungus host cell transformation using enzyme for the steroid production, flavor and electro-optic compound

L8 ANSWER 62 OF 63 FROSTI COPYRIGHT 2003 LFRA
TI Genes of carotenoid biosynthesis and metabolism and methods of use therof.

L8 ANSWER 63 OF 63 FROSTI COPYRIGHT 2003 LFRA
TI Genes of carotenoid biosynthesis and metabolism and methods of use therof.

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(FILE 'HOME' ENTERED AT 17:08:45 ON 04 JUN 2003)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 17:09:01 ON 04 JUN 2003

SEA LYCOPE? AND CYCLAS?

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2	FILE ANABSTR
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L1 QUE LYCOPE? AND CYCLAS?

FILE 'DGENE, CAPLUS, USPATFULL, GENBANK, BIOSIS, SCISEARCH, MEDLINE, BIOTECHNO, ESBIOBASE, CABA, EMBASE, LIFESCI, BIOTECHDS, WPIDS, AGRICOLA, PASCAL, FROSTI, TOXCENTER, IFIPAT' ENTERED AT 17:11:30 ON 04 JUN 2003

L2 1275 S LYCOPE? (S) CYCLAS?

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L3          982 S L2 (S) CAROTE?
L4          298 S L3 (S) EPSIL?
L5          216 DUP REM L4 (82 DUPLICATES REMOVED)
L6            4 S L5 (S) ADON?
L7            2 S L5 (S) PALAES?
L8           63 S L5 (S) (RECOMBIN? OR CLON? OR ISOL?)

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COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                               52.54      54.95

 SESSION WILL BE HELD FOR 60 MINUTES
STN INTERNATIONAL SESSION SUSPENDED AT 17:19:25 ON 04 JUN 2003
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US PATENT & TRADEMARK OFFICE
PATENT APPLICATION FULL TEXT AND IMAGE DATABASE

NO DP

United States Patent Application *20020086380*
Kind Code **A1**
CUNNINGHAM JR, FRANCIS X. **July 4, 2002**

GENES ENCODING EPSILON LYCOPENE CYCLASE AND METHOD FOR PRODUCING BICYCLIC CAROTENE

Abstract

The present invention relates to the DNA sequence for eukaryotic genes encoding .epsilon. cyclase isolated from romaine lettuce as well as vectors containing the same and hosts transformed with said vectors. The present invention provides methods for controlling the ratio of various carotenoids in a host and to the production of novel carotenoid pigments. The present invention also provides a method for treating disease by administering carotenoids obtained from transformed hosts, or by administering a composition containing the transformed hosts.

Inventors: **CUNNINGHAM JR, FRANCIS X.**; *(CHEVY CHASE, MD)*

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US

Serial No.: **084222**
Series Code: **09**
Filed: **May 26, 1998**

U.S. Current Class:	**435/183**; 435/232; 435/252.3; 435/320.1; 435/410; 435/411; 514/763; 585/23
U.S. Class at Publication:	**435/183**; 435/232; 435/320.1; 435/252.3; 435/410; 435/411; 514/763; 585/23
Intern'l Class:	C12N 009/00

Claims

What is claimed as new and is desired to be secured by Letters Patent of the United States is:

1. An isolated eukaryotic enzyme which converts lycopene to epsilon, epsilon-carotene.

2. An isolated eukaryotic enzyme of claim 1 having the amino acid sequence of SEQ ID NO: 2.

3. An isolated DNA sequence comprising a gene encoding the eukaryotic .epsilon. cyclase of claim 2.

4. The isolated DNA sequence according to claim 3, having the nucleic acid sequence of SEQ ID NO: 1.

5. An expression vector comprising the DNA sequence of claim 3.

6. A host containing the expression vector of claim 5.

7. The host of claim 6, wherein said host is E. coli.

8. The host of claim 6, wherein said host is a plant.

9. The host of claim 8, wherein said host is marigold.

10. The host of claim 8, wherein said host is tomato.

11. A composition comprising the host of claim 6.

12. A composition comprising the host of claim 8.

13. A composition comprising bicyclic epsilon carotene obtained from the host of claim 6.

14. A composition comprising bicyclic epsilon carotene obtained from the host of claim 8.

15. A method for treating disease comprising administering to a patient in need thereof, an amount of the composition of claim 13 sufficient to treat said disease.

16. A method for treating disease comprising administering to a patient in need thereof, an amount of the composition of claim 14 sufficient to treat said disease.

Description

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present invention describes the DNA sequence for eukaryotic genes encoding .epsilon. lycopene cyclase as well as vectors containing the same and hosts transformed with these vectors. The present invention also provides a method for augmenting the accumulation of carotenoids and production of novel and rare carotenoids. The present invention provides methods for controlling the ratio of various carotenoids in a host. Additionally, the present invention provides a method for screening for eukaryotic genes encoding enzymes of carotenoid biosynthesis and metabolism. The invention also provides transgenic plants having therapeutic properties, methods for preparing a therapeutic composition, and methods for treating disease by administering the therapeutic plants and compositions.

DISCUSSION OF THE BACKGROUND

[0002] Carotenoid pigments with cyclic endgroups are essential components of the photosynthetic apparatus in oxygenic photosynthetic organisms (e.g., cyanobacteria, algae and plants; Goodwin, 1980). The symmetrical bicyclic yellow carotenoid pigment .beta.-carotene (or, in rare cases, the asymmetrical bicyclic .alpha.-carotene) is intimately associated with the photosynthetic reaction centers and plays a vital role in protecting against potentially lethal photooxidative damage (Koyama, 1991). .beta.-carotene and other carotenoids derived from it or from .alpha.-carotene also serve as light-harvesting pigments (Siefermann-Harms, 1987), are involved in the thermal dissipation of excess light energy captured by the light-harvesting antenna (Demmig-Adams & Adams, 1992), provide substrate for the biosynthesis of the plant growth regulator abscisic acid (Rock & Zeevaart, 1991; Parry & Horgan, 1991), and are precursors of vitamin A in human and animal diets (Krinsky, 1987). Plants also exploit carotenoids as coloring agents in flowers and fruits to attract pollinators and agents of seed dispersal (Goodwin, 1980). The color provided by carotenoids is also of agronomic value in a number of important crops. Carotenoids are currently harvested from plants for use as pigments in food and feed.

[0003] Two types of cyclic endgroups are commonly found in higher plant carotenoids, these are referred to as the .beta. and .epsilon. cyclic endgroups (FIG. 2; the acyclic endgroup is referred to as the .PSI. or psi endgroup). These cyclic endgroups differ only in the position of the double bond in the ring. Carotenoids with two .beta. rings are ubiquitous, and those with one .beta. and one .epsilon. ring are common, but carotenoids with two .epsilon. rings are found in significant amounts in relatively few plants. .beta.-Carotene (FIG. 1) has two .beta. endgroups and is a symmetrical compound that is the precursor of a number of other important plant carotenoids such as zeaxanthin and violaxanthin (FIG. 1).

[0004] Carotenoid enzymes have previously been isolated from a variety of sources including bacteria (Armstrong et al., 1989, Mol. Gen. Genet. 216, 254-268; Misawa et al., 1990, J. Bacteriol., 172, 6704-12), fungi (Schmidhauser et al., 1990, Mol. Cell. Biol. 10, 5064-70), cyanobacteria (Chamovitz et al., 1990, Z. Naturforsch, 45c, 482-86) and higher plants (Bartley et al., Proc. Natl. Acad. Sci USA 88, 6532-36; Martinez-Ferez & Vioque, 1992, Plant Mol. Biol. 18, 981-83). Many of the isolated enzymes show a great diversity in function and inhibitory properties between sources. For example, phytoene desaturases from Synechococcus and higher plants carry out a two-step desaturation to yield .zeta.-carotene as a reaction product; whereas the same enzyme from Erwinia introduces four double bonds forming lycopene. Similarity of the amino acid sequences are very low for bacterial versus plant enzymes. Therefore, even with a gene in hand from one source, it is difficult to screen for a gene with similar function in another source. In particular, the sequence similarity between bacterial/fungal and cyanobacterial/plants genes is quite low.

[0005] The difficulties in isolating related genes is exemplified by recent efforts to isolated the enzyme

which catalyzes the formation of .beta.-carotene from the acyclic precursor lycopene. Although this enzyme had been isolated in a bacterium, prior to the invention described in U.S. Ser. No. 08/142,195 (which is hereby incorporated by reference in its entirety), it had not been isolated from any photosynthetic organism nor had the corresponding genes been identified and sequenced or the cofactor requirements established. The isolation and characterization of the enzyme catalyzing formation of .beta.-carotene in the cyanobacterium Synechococcus PCC7942 was described by Cunningham et al. in 1993 and 1994.

[0006] The .beta.-cyclase of Arabidopsis adds two rings to the symmetrical lycopene to form the bicyclic .beta.-carotene, but the related .epsilon.-cyclase of Arabidopsis, which has 36% identity for the predicted amino acid sequences) adds only a single ring to form the monocyclic .delta.-carotene (Cunningham et al, 1996, Plant Cell 8:1613-1626; U.S. application Ser. No. 08/624,125 filed Mar. 29, 1996, which is incorporated by reference herein in its entirety). These differences in function provide a simple mechanism for adjusting the proportions of .beta.,.beta.-and .beta., .epsilon.-carotenoids while at the same time preventing formation of carotenoids with two epsilon rings.

[0007] In view of the afore-mentioned deficiencies with prior art methods of producing carotenoids with two epsilon rings, it is clear that there exists a need in the art for such methods.

SUMMARY OF THE INVENTION

[0008] Accordingly, a first object of this invention is to provide isolated eukaryotic genes which encode enzymes which encode lycopene epsilon cyclases which form bicyclic epsilon-carotene.

[0009] A second object of the present invention is to provide vectors containing said genes.

[0010] A third object of the present invention is to provide hosts transformed with said vectors.

[0011] A further object is to provide a method for producing a lycopene epsilon cyclase using the transformed host.

[0012] A still further object is to provide the lycopene epsilon cyclase so produced.

[0013] Another object of the present invention is to provide hosts which accumulates novel or rare carotenoids or which overexpress known carotenoids.

[0014] Yet another object of the invention is to provide a method for producing novel or rare carotenoids.

[0015] Another object of this invention is to secure the expression of eukaryotic carotenoid-related genes in a recombinant prokaryotic host.

[0016] An additional object of the invention is a method of preparing a therapeutic composition comprising either the host cell which expresses the lycopene epsilon cyclase or the isolated carotenoids produced by the host cell containing the lycopene epsilon cyclase.

[0017] Another object of the invention is to provide a method for the treatment of disease by providing to a patient in need thereof, an amount of the rare carotenoids in an amount sufficient to treat the disease.

[0018] These and other objects of the present invention have been realized by the present inventors as described below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] A more complete appreciation of the invention and many of the attendant advantages thereof will be readily obtained as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings, wherein:

[0020] FIG. 1 depicts possible routes of synthesis of cyclic carotenoids and some common plant and algal xanthophylls (oxycarotenolds) from lycopene. Activities of the .epsilon.-cylase enzyme of lettuce are indicated by bold arrows labelled with .epsilon.. The reaction leading to .epsilon.-carotene from .delta.-carotene is not catalyzed by the lycopene .epsilon. cyclase of Arabidopsis (Cunningham 1996; U.S. Ser. No. 08/624,125) or other known .epsilon.-cyclases. Therefore, formation of .epsilon.-cartene and carotenoids derived from it is now made possible with the lettuce lycopene .epsilon.-cyclase describe herein. Arrows labelled with .beta. indicate reactions synthesize by .beta.-cyclase.

[0021] FIG. 2 depicts the carotene endgroups which are commonly found in plants.

[0022] FIG. 3 is a DNA sequence of the romaine lettuce cDNA (SEQ ID NO:1) encoding lycopene epsilon cyclase.

[0023] FIG. 4 is the predicted amino acid sequence of the romaine lettuce lycopene epsilon cyclase (SEQ ID NO:2).

[0024] FIG. 5 is a comparison between the predicted amino acid sequences of romaine lettuce (from clone DY4; SEQ ID NO:2) and Arabidopsis (from clone y2; SEQ ID NO:3) lycopene epsilon cyclase.

[0025] FIG. 6 shows the nucleotide and amino acid sequences of the .epsilon.-cyclase #3 of Adonis palaestina, which also forms bicyclic epsilon caratene.

[0026] FIG. 7 Shows a sequence comparison of the Adonis palaestina .epsilon.-cyclase #3 compared to the Adonis palaestina .epsilon.-cyclase #5, the latter of which adds only a single epsilon ring to lycopene. Five amino acid differences are noted, which may be targets for site-directed mutagenesis to form the lycopene .epsilon.-cyclase which adds two .epsilon. rings to lycopene.

DETAILED DESCRIPTION

[0027] Romaine lettuce is one of the rare plant species that produces an abundance of a carotenoid with two epsilon rings (lactucaxanthin). The present inventors have isolated a gene encoding the epsilon cyclase from this plant, and have found that it is similar in sequence to that of Arabidopsis (about 65% identity). However, the lettuce enzyme efficiently adds two epsilon rings to lycopene to form the bicyclic epsilon-carotene.

[0028] The present invention also relates to methods for transforming known carotenoids into novel or rare products. That is, currently .epsilon.-carotene (see FIG. 1) and .gamma.-carotene can only be isolated in minor amounts. As described below, the enzymes of the invention can be produced and used to transform lycopene to bicyclic .epsilon.-carotene. With such a product in hand, bulk biosynthesis of other carotenoids derived from the bicyclic epsilon carotene are possible.

[0029] The eukaryotic genes in the carotenoid biosynthetic pathway differ from their prokaryotic counterparts in their 5' region. As used herein, the 5' region is the region of eukaryotic DNA which precedes the initiation codon of the counterpart gene in prokaryotic DNA. That is, when the consensus areas of eukaryotic and prokaryotic genes are aligned, the eukaryotic genes contain additional coding sequences upstream of the prokaryotic initiation codon.

[0030] The invention also relates to genes encoding lycopene epsilon cyclase which are truncated at the 5' region of the gene. Preferably, such truncated genes are truncated to within 0-50, preferably 0-25, codons of the 5' initiation codon of their prokaryotic counterparts as determined by alignment maps.

[0031] In addition to novel enzymes produced by truncating the 5' region of known enzymes, novel enzymes which can participate in the formation of novel carotenoids can be formed by replacing portions of one gene with an analogous sequence from a structurally related gene. The information for adding two epsilon rings can be found in the 3' half of the romaine lettuce gene. Thus, one example of such a hybrid gene construct would include the first half of the romaine lettuce cyclase gene in combination with the second (3') half of another plant cyclase gene, such as the potato gene or by random of site directed mutagenesis of a mono-.epsilon. cyclase.

[0032] Vectors

[0033] The genes encoding the carotenoid enzymes as described above, when cloned into a suitable expression vector, can be used to overexpress these enzymes in a plant expression system or to inhibit the expression of these enzymes. The production or the biochemical activity of the product of epsilon-cyclase genes and cDNAs may be reduced or inhibited by a number of different approaches available to those skilled in the art [including but not limited to such methodologies or approaches as anti-sense (e.g., Gray et al (1992) Plant Mol. Biol. 19:69-87), ribozymes (e.g., Wegener et al (1994) Mol. Gen. Genet. 245:465-470), co-suppression (e.g., Fray and Grierson (1993) Plant Mol. Biol. 22:589-602), targeted disruption of the gene (e.g., Schaefer et al. (1997) Plant J. 11:1195-1206), intracellular antibodies (e.g., Rondon and Marasco (1997) Ann. Rev. Microbiol. 51:257-283 or whatever other approaches rely on the knowledge or availability of the gene, cDNA, or polypeptide and/or the sequences of these] to thereby reduce accumulation of carotenoids with psilon rings and compounds derived from them.

[0034] For example, a vector containing the gene encoding .epsilon.-cyclase can be used to increase the amount of bicyclic epsilon-carotene in an organism and thereby alter the nutritional value, pharmacology and visual appearance value of the organism. In addition, the transformed organism can be used in the formulation of therapeutic agents, for example in the treatment of cancer (Mayne et al (1996) FASEB J. 10:690-701; Tsushima et al (1995) Biol. Pharm. Bull. 18:227-233, which are both incorporated herein by reference in their entireties).

[0035] In a preferred embodiment, the vectors of the present invention contain a DNA encoding an eukaryotic IPP isomerase upstream of a DNA encoding a second eukaryotic carotenoid enzyme. The inventors have discovered that inclusion of an IPP isomerase gene increases the supply of substrate for the carotenoid pathway; thereby enhancing the production of carotenoid endproducts. This is apparent from the much deeper pigmentation in carotenoid-accumulating colonies of E. coli which also contain one of the aforementioned IPP isomerase genes when compared to colonies that lack this additional IPP isomerase gene. Similarly, a vector comprising an IPP isomerase gene can be used to enhance production of secondary metabolites of dimethylallyl pyrophosphate (such as isoprenoids, steroids, carotenoids, etc.).

[0036] Alternatively, an anti-sense strand of one of the above genes can be inserted into a vector. For example, the .epsilon.-cyclase gene can be inserted into a vector and incorporated into the genomic DNA of a host, thereby inhibiting the synthesis of .epsilon.,.beta. carotenoids (lutein and .alpha.-carotene) and enhancing the synthesis of bicyclic epsilon carotenoids.

[0037] Suitable vectors according to the present invention comprise a eukaryotic gene encoding an enzyme involved in carotenoid biosynthesis or metabolism and a suitable promoter for the host can be constructed using techniques well known in the art (for example Sambrook et al., Molecular Cloning A Laboratory

Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989).

[0038] Suitable vectors for eukaryotic expression in plants are described in Frey et al., Plant J. (1995) 8(5):693 and Misawa et al, 1994; incorporated herein by reference in their entireties.

[0039] Suitable vectors for prokaryotic expression include pACYC184, pUC119, and pBR322 (available from New England BioLabs, Bevery, Mass.), pTrcHis (Invitrogen), Bluescript SK (Stratagene) and pET28 (Novagen) and derivatives thereof.

[0040] The vectors of the present invention can additionally contain regulatory elements such as promoters, repressors selectable markers such as antibiotic resistance genes, etc.

[0041] Hosts

[0042] Host systems according to the present invention can comprise any organism that already produces carotenoids or which has been genetically modified to produce carotenoids.

[0043] Organisms which already produce carotenoids include plants, algae, some yeasts, fungi and cyanobacteria and other photosynthetic bacteria. Transformation of these hosts with vectors according to the present invention can be done using standard techniques such as those described in Misawa et al., (1990) supra; Hundle et al., (1993) supra; Hundle et al., (1991) supra; Misawa et al., (1991) supra; Sandmann et al., supra; and Schnurr et al., supra; all incorporated herein by reference in their entireties.

[0044] E. coli is an example of one type of bacteria which is suitable as a host for expression of the present enzymes (Cunningham et al, (1996) The Plant Cell 8:1613-1626, which is incorporated herein by reference in its entirety). A vector is used to construct plasmids containing genes encoding the enzymes of the invention, which vector allows it to coexist in E. coli with cloning vectors that contain the more common ColE1 origin of replication. The addition of epsilon cyclic end groups to the pink-colored lycopene will result in products that are yellow or orange-yellow in color. Therefore, the functioning of the epsilon lycopene cyclase of the invention may be detected by a change in the color of E. coli cultures that accumulate lycopene. Such assays are termed color complementation assays.

[0045] Alternatively, transgenic organisms can be constructed which include the DNA sequences of the present invention (Bird et al, 1991; Bramley et al, 1992; Misawa et al, 1994a; Misawa et al, 1994b; Cunningham et al, 1993, all of which are incorporated by reference herein in their entireties). The incorporation of these sequences can allow the controlling of carotenoid biosynthesis, content, or composition in the host cell. These transgenic systems can be constructed to incorporate sequences which allow over-expression of the carotenoid genes of the present invention. Transgenic systems can also be constructed containing antisense expression of the DNA sequences of the present invention. Such antisense expression would result in the accumulation of the substrates of the enzyme encoded by the sense strand.

[0046] Appropriate transgenic hosts include lettuce, the natural host, but also other plants such as marigold, tomato, pepper, banana, potato and the like. Essentially any plant is suitable for expressing the present enzyme, but the preferred plants are those which already produce high levels of carotenoids, and those which are normally ingested as foods or used as a source of carotenoid pigments. In particular, plants which bear fruit can be manipulated in such a way as to provide tissue-specific expression in fruit. Marigold is a particularly preferred host, because it can be used as a "bioreactor" for bulk production of carotenoids, and is actually grown commercially as a carotenoid source for chicken feed. For expression in marigold, a promoter can be used which is "flower-specific." Another preferred transgenic plant is tomato, because this plant already produces high levels of lycopene. Indeed, it has been reported that there is a correlation between consuming tomatoes and decreased incidence of colon cancer (mayne, supra).

[0047] A Method for Screening for Eukaryotic Genes which Encode Enzymes Involved in Carotenoid Biosynthesis

[0048] The method of the present invention comprises transforming a prokaryotic host with a DNA which may contain a eukaryotic or prokaryotic carotenoid biosynthetic gene; culturing said transformed host to obtain colonies; and screening for colonies exhibiting a different color than colonies of the untransformed host.

[0049] Suitable hosts include E. coli, cyanobacteria such as Synechococcus and Synechocystis, alga and plant cells. E. coli are preferred.

[0050] In a preferred embodiment, the above "color complementation test" can be enhanced by using mutants which are either (1) deficient in at least one carotenoid biosynthetic gene or (2) overexpress at least one carotenoid biosynthetic gene. In either case, such mutants will accumulate carotenoid precursors.

[0051] Prokaryotic and eukaryotic genomic and cDNA libraries can be screened in total for the presence of genes of carotenoid biosynthesis, metabolism and degradation. Preferred organisms to be screened include photosynthetic organisms, humans and animals.

[0052] E. coli can be transformed with these eukaryotic cDNA libraries using conventional methods such as those described in Sambrook et al, 1989 and according to protocols described by the venders of the cloning vectors.

[0053] For example, the cDNA libraries in bacteriophage vectors such as lambdaZAP (Stratagene) or lambdaZIPLOX (Gibco BRL) can be excised en masse and used to transform E. coli. Suitable vectors include pACYC184, pUC119, pBR322 (available from New England BioLabs, Bevery, Mass.). pACYC is preferred.

[0054] Transformed E. coli can be cultured using conventional techniques. The culture broth preferably contains antibiotics to select and maintain plasmids. Suitable antibiotics include penicillin, ampicillin, chloramphenicol, etc. Culturing is typically conducted at 15-45.degree. C., preferably at room temperature (16-28.degree. C.), for 12 hours to 7 days.

[0055] Cultures are plated and the plates are screened visually for colonies with a different color than the colonies of the host E. coli transformed with the empty vector. For example, E. coli transformed with the plasmid, pAC-BETA (described below), produce yellow colonies that accumulate .beta.-carotene. After transformation with a cDNA library, colonies which contain a different hue than those formed by E. coli/pAC-BETA would be expected to contain enzymes which modify the structure or degree of expression of .beta.-carotene. Similar standards can be engineered which overexpress earlier products in carotenoid biosynthesis, such as lycopene, .gamma.-carotene, etc.

[0056] Having generally described this invention, a further understanding can be obtained by reference to certain specific examples which are provided herein for purposes of illustration only and are not intended to be limiting unless otherwise specified.

EXAMPLE

[0057] Isolation of Lycopene Epsilon Cyclase

[0058] The lycopene epsilon cyclase was isolated from a romaine lettuce library obtained from Dr. Harry Y.

Yamamoto (University of Hawaii, Honolulu) essentially as disclosed in Cunningham et al, 1996, supra, and Bugos and Yamamoto (1996) Proc. Natl. Acad. Sci. USA 93:6320-6325, both of which are incorporated herein by reference in their entireties. Functional clones were identified by the color complementation test.

[0059] Pigment Analysis

[0060] A single colony was used to inoculate 50 ml of LB containing ampicillin and chloramphenicol in a 250-ml flask. Cultures were incubated at 28.degree. C. for 36 hours with gentle shaking, and then harvested at 5000 rpm in an SS-34 rotor. The cells were washed once with distilled H.sub.2O and resuspended with 0.5 ml of water. The extraction procedures and HPLC were essentially as described previously (Cunningham et al, 1994).

[0061] Organisms and Growth Conditions

[0062] E. coli strains TOP10 and TOP10 F' (obtained from Invitrogen Corporation, San Diego, Calif.) and XL1-Blue (Stratagene) were grown in Luria-Bertani (LB) medium (Sambrook et al., 1989) at 37.degree. C. in darkness on a platform shaker at 225 cycles per min. Media components were from Difco (yeast extract and tryptone) or Sigma (NaCl). Ampicillin at 150 .mu.g/mL and/or chloramphenicol at 50 .mu.g/mL (both from United States Biochemical Corporation) were used, as appropriate, for selection and maintenance of plasmids.

[0063] Mass Excision and Color Complementation Screening of Romaine Lettuce cDNA Library

[0064] A cDNA library of romaine lettuce in lambda ZAPII (Bugos & Yamamoto) was obtained from Henry Yamamoto, as noted above. An aliquot of each library was treated to cause a mass excision of the cDNAs and thereby produce a phagemid library according to the instructions provided by the supplier of the cloning vector (Stratagene; E. coli strain XL1-Blue and the helper phage R408 were used). The titre of the excised phagemid was determined and the library was introduced into a lycopene-accumulating strain of E. coli TOP10 F' by incubation of the phagemid with the E. coli cells for 15 min at 37.degree. C. Cells had been grown overnight at 30.degree. C. in LB medium supplemented with 2% (w/v) maltose and 10 mM MgSO.sub.4 (final concentration), and harvested in 1.5 ml microfuge tubes at a setting of 3 on an Eppendorf microfuge (5415C) for 10 min. The pellets were resuspended in 10 mM MgSO.sub.4 to a volume equal to one-half that of the initial culture volume. Transformants were spread on large (150 mm diameter) LB agar petri plates containing antibiotics to provide for selection of cDNA clones (ampicillin) and maintenance of pAC-LYC (chloramphenicol). Approximately 10,000 colony forming units were spread on each plate. Petri plates were incubated at room temperature for 2 to 7 days to allow maximum color development. Plates were screened visually with the aid of an illuminated 3.times.magnifier and a low power stage-dissecting microscope for the rare, pale pinkish-yellow to deep-yellow colonies that could be observed in the background of pink colonies. A colony color of yellow or pinkish-yellow was taken as presumptive evidence of a cyclization activity. These yellow colonies were collected with sterile toothpicks and used to inoculate 3 ml of LB medium in culture tubes with overnight growth at 37.degree. C. and shaking at 225 cycles/min. Cultures were split into two aliquots in microfuge tubes and harvested by centrifugation at a setting of 5 in an Eppendorf 5415C microfuge. After discarding the liquid, one pellet was frozen for later purification of plasmid DNA. To the second pellet was added 1.5 ml EtOH, and the pellet was resuspended by vortex mixing, and extraction was allowed to proceed in the dark for 15-30 min with occasional remixing. Insoluble materials were pelleted by centrifugation at maximum speed for 10 min in a microfuge. Absorption spectra of the supernatant fluids were recorded from 350-550 nm with a Perkin Elmer lambda six spectrophotometer.

[0065] Analysis of Isolated Clones

[0066] Eight of the yellow colonies contained .epsilon.-carotene indicating that a single gene product catalyzes both cyclizations required to form the two .epsilon. endgroups of the symmetrical .epsilon.-carotene from the symmetrical precursor lycopene.

[0067] The availability of the romaine lettuce gene encoding the .epsilon. cyclase enables the directed manipulation of plant and algal species for modification of carotenoid content and composition. Through inactivation of the .epsilon. cyclase, whether at the gene level by deletion of the gene or by insertional inactivation or by reduction of the amount of enzyme formed (by such as antisense technology), one may increase the formation of .beta.-carotene and other pigments derived from it. Since vitamin A is derived only from carotenoids with .beta. endgroups, an enhancement of the production of .beta.-carotene versus .alpha.-carotene may enhance nutritional value of crop plants. Reduction of carotenoids with .epsilon. endgroups may also be of value in modifying the color properties of crop plants and specific tissues of these plants. Alternatively, where production of .alpha.-carotene, or pigments such as lutein that are derived from .alpha.-carotene, is desirable, whether for the color properties, nutritional value or other reason, one may overexpress the .epsilon. cyclase or express it in specific tissues. Wherever agronomic value of a crop is related to pigmentation provided by carotenoid pigments the directed manipulation of expression of the .epsilon. cyclase gene and/or production of the enzyme may be of commercial value.

[0068] The predicted amino acid sequence of the romaine lettuce .epsilon. cyclase enzyme (SEQ ID NO:2) was determined. A comparison of the amino acid sequences of the .epsilon. cyclase enzymes of Arabidopsis thaliana and romaine lettuce (FIG. 5) as predicted by the DNA sequence of the respective genes (FIG. 3 for the .epsilon. cyclase cDNA sequence), indicates that these two enzymes have many regions of sequence similarity, but they are only about 65% identical overall at the amino acid level.

[0069] References

[0070] Bird et al, 1991 Biotechnology 9, 635-639.

[0071] Bishop et al., (1995) FEBS Lett. 367, 158-162.

[0072] Bramley, P. M. (1985) Adv. Lipid Res. 21, 243-279.

[0073] Bramley, P. M. (1992) Plant J. 2, 343-349.

[0074] Britton, G. (1988). Biosynthesis of carotenoids. In Plant Pigments, T. W. Goodwin, ed. (London: Academic Press), pp. 133-182.

[0075] Britton, G. (1979) Z. Naturforsch. Section C Biosci. 34, 979-985.

[0076] Britton, G. (1995) UV/Visible spectroscopy. In Carotenoids, Vol. IB: Spectroscopy, G. Britton, S. Liaaen-Jensen, H. P. Pfander, eds. (Basel: Birkhauser Verlag), pp. 13-62.

[0077] Bouvier et al., (1994) Plant J. 6, 45-54.

[0078] Cunningham et al., (1985) Photochem. Photobiol. 42: 295-307.

[0079] Cunningham et al., (1993) FEBS Lett. 328, 130-138.

[0080] Cunningham et al., (1994) Plant Cell 6, 1107-1121.

[0081] Davies, B. H. (1976). Carotenoids. In Chemistry and Biochemistry of Plant Pigments, Vol. 2, T. W.

Goodwin, ed (New York: Academic Press), pp. 38-165.

[0082] Del Sal et al., (1988). Nucl. Acids Res. 16, 9878.

[0083] Demmig-Adams & Adams, (1992) Ann. Rev. Plant Physiol. Mol. Biol. 43, 599-626.

[0084] Enzell & Back, (1995) Mass spectrometry. In Carotenoids, Vol. IB: Spectroscopy, G. Britton, S. Liaaen-Jensen, H. P. Pfander, eds. (Basel: Birkhauser Verlag), pp. 261-320.

[0085] Frank & Cogdell (1993) Photochemistry and function of carotenoids in photosynthesis. In Carotenoids in Photosynthesis. A. Young and G. Britton, eds. (London: Chapman and Hall). pp. 253-326.

[0086] Goodwin, T. W. (1980). The Biochemistry of the Carotenoids. 2nd ed, Vol. 1 (London: Chapman and Hall.

[0087] Horvath et al., (1972) Phytochem. 11, 183-187.

[0088] Hugueney et al., (1995) Plant J. 8, 417-424.

[0089] Hundle et al., (1991) Photochem. Photobiol. 54, 89-93.

[0090] Jensen & Jensen, (1971) Methods Enzymol. 23, 586-602.

[0091] Kargl & Quackenbush, (1960) Archives Biochem. Biophys. 88, 59-63.

[0092] Kargl et al., (1960) Proc. Am. Hort. Soc. 75, 574-578.

[0093] Kieber et al., (1993) Cell 72, 427-441.

[0094] Koyama, Y. (1991) J. Photochem. Photobiol., B, 9, 265-80.

[0095] Krinsky, N. I. (1987) Medical uses of carotenoids. In Carotenoids, N. I. Krinsky, M. M. Mathews-Roth, and R. F. Taylor, eds. (New York: Plenum), pp. 195-206.

[0096] Kyte & Doolittle, (1982) J. Mol. Biol. 157, 105-132.

[0097] LaRossa & Schloss, (1984) J. Biol. Chem. 259, 8753-8757.

[0098] Misawa et al., (1994a) Plant J. 6, 481-489.

[0099] Misawa et al., (1994b) J. Biochem, Tokyo, 116, 980-985.

[0100] Norris et al., (1995) Plant Cell 7, 2139-2149.

[0101] Pecker et al., (1996) Submitted to Plant Mol. Biol.

[0102] Perry et al., (1986) J. Bacteriol. 168, 607-612.

[0103] Persson & Argos, (199) J. Mol. Biol. 237, 182-192.

[0104] Plumley & Schmidt, (1987) Proc. Nat. Acad. Sci. USA 83, 146-150.

[0105] Plumley & Schmidt, (1995) Plant Cell 7, 689-704.

[0106] Rossmann et al., (1974) Nature 250, 194-199.

[0107] Rock & Zeevaart (1991) Proc. Nat. Acad. Sci. USA 88, 7496-7499.

[0108] Rost et al., (1995) Protein Science 4, 521-533.

[0109] Sambrook et al., (1989) Molecular Cloning: A Laboratory Manual, 2nd edition (Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press).

[0110] Sancar, A. (1994) Biochemistry 33, 2-9.

[0111] Sander & Schneider, (1991) Proteins 9, 56-68.

[0112] Sandmann, G. (1994) Eur. J. Biochem. 223, 7-24.

[0113] Scolnik & Bartley, (1995) Plant Physiol. 108, 1342.

[0114] Siefermann-Harms, D. (1987) Physiol. Plant. 69, 561-568.

[0115] Spurgeon & Porter, (1980). Biosynthesis of carotenoids. In Biochemistry of Isoprenoid Compounds, J. W. Porter, and S. L. Spurgeon, eds. (New York: Wiley), pp. 1-122.

[0116] Tomes, M. L. (1963) Bot. Gaz. 124, 180-185.

[0117] Tomes, M. L. (1967) Genetics 56, 227-232.

[0118] Tuveson et al., (1986) J. Bacteriol. 170, 4675-4680.

[0119] Van Beeumen et al., (1991) J. Biol. Chem. 266, 12921-12931.

[0120] Weedon & Moss, (1995) Structure and Nomenclature. In Carotenoids, Vol. IB: Spectroscopy, G. Britton, S. Liaaen-Jensen, H. P. Pfander, eds. (Basel: Birkhauser Verlag), pp. 27-70.

[0121] Wierenga et al., (1986) J. Mol. Biol. 187, 101-107.

[0122] Zechmeister, L. (1962) Cis-Trans Isomeric Carotenoids, Vitamins A and Arylpolyenes. Springer-Verlag, Vienna.

[0123] Having now fully described the invention, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the invention as set forth herein.

Sequence CWU 1

6 1 1780 DNA romaine lettuce n is an unspecified nucleotide 1 gaaacaaatg acgtgaaagt tcttcaaaat tgaattaatt gtaatcctga aaacttgatt 60 tgtgatagaa gaatcaatgg agtgctttgg agctcgaaac atgacggcaa caatggcggt 120 ttttacgtgc cctagattca cggactgtaa tatcaggcac aaattttcgt tactgaaaca 180 acgaagattt actaatttat cagcatcgtc ttcgttgcgt caaattaagt gcagcgctaa 240 aagcgaccgt tgtgtagtgg ataaacaagg gatttccgta gcagacgaag aagattatgt 300

gaaggccggt ggatcggagc tgtttttttgt tcaaatgcag cggactaagt ccatggaaag 360 ccagtctaaa ctttccgaaa agctagcaca gataccaatt ggaaattgca tacttgatct 420 ggttgtaatc ggttgtggcc ctgctggcct tgctcttgct gcagagtcag ccaaactagg 480 gttgaacgtt ggactcattg gccctgatct tccttttaca aacaattatg gtgtttggca 540 ggatgaattt ataggtcttg gacttgaagg atgcattgaa cattcttgga aagatactct 600 tgtatacctt gatgatgctg atcccatccg cataggtcgt gcatatggca gagttcatcg 660 tgatttactt catgaagagt tgttaagaag gtgtgtggaa tcaggtgttt catatctaag 720 ctccaaagta gaaagaatca ctgaagctcc aaatggctat agtctcattg aatgtgaagg 780 caatatcacc attccatgca ggcttgctac tgttgcatca ggggcagctt cagggaaatt 840 tctggagtat gaacttgggg gtccccgtgt ttgtgtccaa acagcttatg gtatagaggt 900 tgaggttgaa aacaacccct atgatccaga tctaatggtg ttcatggatt atagagactt 960 ctcaaaacat aaaccggaat ctttagaagc aaaatatccg actttcctct atgtcatggc 1020 catgtctcca acaaaaatat tcttcgagga aacttgttta gcttcaagag aagccatgcc 1080 tttcaatctt ctaaagtcca aactcatgtc acgattaaag gcaatgggta tccgaataac 1140 aagaacgtac gaagaggaat ggtcgtatat ccccgtaggt ggatcgttac ctaatacaga 1200 acaaaagaat ctcgcatttg gtgctgcagc tagtatggtg caccctgcca cagggtattc 1260 agttgttcga tctttgtcag aagctcctaa ttatgcagca gtcattgcta agattttaag 1320 acaagatcaa tctaaagaga tgatttctct tggaaaatac actaacattt caaaacaagc 1380 atgggaaaca ttgtggccac ttgaaaggaa aagacagcga gccttctttc tattcggact 1440 atcacacatc gtgctaatng atctagaggg aacacgtaca tttttccgta ctttctttcg 1500 tttgcccaaa tggatgtggt ggggattttt ggggtcttct ttatcttcaa cggatttgat 1560 aatatttgcg ctttatatgt ttgtgatagc acctcacagc ttgagaatgg aactggttag 1620 acatctactt tctgatccga caggggcaac tatggtaaaa gcatatctca ctatatagat 1680 ttagattata taaataatac ccatatcttg catatatata agccttattt atttcttttg 1740 tacccccaca acaacatact cgttaattat atgtttttta 1780 2 533 PRT romaine lettuce 2 Met Glu Cys Phe Gly Ala Arg Asn Met Thr Ala Thr Met Ala Val Phe 1 5 10 15 Thr Cys Pro Arg Phe Thr Asp Cys Asn Ile Arg His Lys Phe Ser Leu 20 25 30 Leu Lys Gln Arg Arg Phe Thr Asn Leu Ser Ala Ser Ser Ser Leu Arg 35 40 45 Gln Ile Lys Cys Ser Ala Lys Ser Asp Arg Cys Val Val Asp Lys Gln 50 55 60 Gly Ile Ser Val Ala Asp Glu Glu Asp Tyr Val Lys Ala Gly Gly Ser 65 70 75 80 Glu Leu Phe Phe Val Gln Met Gln Arg Thr Lys Ser Met Glu Ser Gln 85 90 95 Ser Lys Leu Ser Glu Lys Leu Ala Gln Ile Pro Ile Gly Asn Cys Ile 100 105 110 Leu Asp Leu Val Val Ile Gly Cys Gly Pro Ala Gly Leu Ala Leu Ala 115 120 125 Ala Glu Ser Ala Lys Leu Gly Leu Asn Val Gly Leu Ile Gly Pro Asp 130 135 140 Leu Pro Phe Thr Asn Asn Tyr Gly Val Trp Gln Asp Glu Phe Ile Gly 145 150 155 160 Leu Gly Leu Glu Gly Cys Ile Glu His Ser Trp Lys Asp Thr Leu Val 165 170 175 Tyr Leu Asp Asp Ala Asp Pro Ile Arg Ile Gly Arg Ala Tyr Gly Arg 180 185 190 Val His Arg Asp Leu Leu His Glu Glu Leu Leu Arg Arg Cys Val Glu 195 200 205 Ser Gly Val Ser Tyr Leu Ser Ser Lys Val Glu Arg Ile Thr Glu Ala 210 215 220 Pro Asn Gly Tyr Ser Leu Ile Glu Cys Glu Gly Asn Ile Thr Ile Pro 225 230 235 240 Cys Arg Leu Ala Thr Val Ala Ser Gly Ala Ala Ser Gly Lys Phe Leu 245 250 255 Glu Tyr Glu Leu Gly Gly Pro Arg Val Cys Val Gln Thr Ala Tyr Gly 260 265 270 Ile Glu Val Glu Val Glu Asn Asn Pro Tyr Asp Pro Asp Leu Met Val 275 280 285 Phe Met Asp Tyr Arg Asp Phe Ser Lys His Lys Pro Glu Ser Leu Glu 290 295 300 Ala Lys Tyr Pro Thr Phe Leu Tyr Val Met Ala Met Ser Pro Thr Lys 305 310 315 320 Ile Phe Phe Glu Glu Thr Cys Leu Ala Ser Arg Glu Ala Met Pro Phe 325 330 335 Asn Leu Leu Lys Ser Lys Leu Met Ser Arg Leu Lys Ala Met Gly Ile 340 345 350 Arg Ile Thr Arg Thr Tyr Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly 355 360 365 Gly Ser Leu Pro Asn Thr Glu Gln Lys Asn Leu Ala Phe Gly Ala Ala 370 375 380 Ala Ser Met Val His Pro Ala Thr Gly Tyr Ser Val Val Arg Ser Leu 385 390 395 400 Ser Glu Ala Pro Asn Tyr Ala Ala Val Ile Ala Lys Ile Leu Arg Gln 405 410 415 Asp Gln Ser Lys Glu Met Ile Ser Leu Gly Lys Tyr Thr Asn Ile Ser 420 425 430 Lys Gln Ala Trp Glu Thr Leu Trp Pro Leu Glu Arg Lys Arg Gln Arg 435 440 445 Ala Phe Phe Leu Phe Gly Leu Ser His Ile Val Leu Met Asp Leu Glu 450 455 460 Gly Thr Arg Thr Phe Phe Arg Thr Phe Phe Arg Leu Pro Lys Trp Met 465 470 475 480 Trp Trp Gly Phe Leu Gly Ser Ser Leu Ser Ser Thr Asp Leu Ile Ile 485 490 495 Phe Ala Leu Tyr Met Phe Val Ile Ala Pro His Ser Leu Arg Met Glu 500 505 510 Leu Val Arg His Leu Leu Ser Asp Pro Thr Gly Ala Thr Met Val Lys 515 520 525 Ala Tyr Leu Thr Ile 530 3 524 PRT Arabidopsis 3 Met Glu Cys Val Gly Ala Arg Asn Phe Ala Ala Met Ala Val Ser Thr 1 5 10 15 Phe Pro Ser Trp Ser Cys Arg Arg Lys Phe Pro Val Val Lys Arg Tyr 20 25 30 Ser Tyr Arg Asn Ile Arg Phe Gly Leu Cys Ser Val Arg Ala Ser Gly 35 40 45 Gly Gly Ser Ser Gly Ser Glu Ser Cys Val Ala Val Arg Glu Asp Phe 50 55 60 Ala Asp Glu Glu Asp Phe Val Lys Ala Gly Gly Ser Glu Ile Leu Phe 65 70 75 80 Val Gln Met Gln Gln Asn Lys Asp Met Asp Glu Gln Ser Lys Leu Val 85 90 95 Asp Lys Leu Pro Pro Ile Ser Ile Gly Asp Gly Ala Leu Asp His Val 100 105 110 Val Ile Gly Cys Gly Pro Ala Gly Leu Ala Leu Ala Ala Glu Ser Ala 115 120 125 Lys Leu Gly Leu Lys Val Gly Leu Ile Gly Pro Asp Leu Pro Phe Thr 130 135 140 Asn Asn Tyr Gly Val Trp Glu Asp Glu Phe Asn Asp Leu Gly Leu Gln 145 150 155 160 Lys Cys Ile Glu His Val Trp Arg Glu Thr Ile Val Tyr Leu Asp Asp 165 170 175 Asp Lys Pro Ile Thr Ile Gly Arg

Ala Tyr Gly Arg Val Ser Arg Arg 180 185 190 Leu Leu His Glu Glu Leu Leu Arg Arg Cys Val Glu Ser Gly Val Ser 195 200 205 Tyr Leu Ser Ser Lys Val Asp Ser Ile Thr Glu Ala Ser Asp Gly Leu 210 215 220 Arg Leu Val Ala Cys Asp Asp Asn Asn Val Ile Pro Cys Arg Leu Ala 225 230 235 240 Thr Val Ala Ser Gly Ala Ala Ser Gly Lys Leu Leu Gln Tyr Glu Val 245 250 255 Gly Gly Pro Arg Val Cys Val Gln Thr Ala Tyr Gly Val Glu Val Glu 260 265 270 Val Glu Asn Ser Pro Tyr Asp Pro Asp Gln Met Val Phe Met Asp Tyr 275 280 285 Arg Asp Tyr Thr Asn Glu Lys Val Arg Ser Leu Glu Ala Glu Tyr Pro 290 295 300 Thr Phe Leu Tyr Ala Met Pro Met Thr Lys Ser Arg Leu Phe Phe Glu 305 310 315 320 Glu Thr Cys Leu Ala Ser Lys Asp Val Met Pro Phe Asp Leu Leu Lys 325 330 335 Thr Lys Leu Met Leu Arg Leu Ser Thr Leu Gly Ile Arg Ile Leu Lys 340 345 350 Thr Tyr Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro 355 360 365 Asn Thr Glu Gln Lys Asn Leu Ala Phe Gly Ala Ala Ala Ser Met Val 370 375 380 His Pro Ala Thr Gly Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro 385 390 395 400 Lys Tyr Ala Ser Val Ile Ala Glu Ile Leu Arg Glu Glu Thr Thr Lys 405 410 415 Gln Ile Asn Ser Asn Ile Ser Arg Gln Ala Trp Asp Thr Leu Trp Pro 420 425 430 Pro Glu Arg Lys Arg Gln Arg Ala Phe Phe Leu Phe Gly Leu Ala Leu 435 440 445 Ile Val Gln Phe Asp Thr Glu Gly Ile Arg Ser Phe Phe Arg Thr Phe 450 455 460 Phe Arg Leu Pro Lys Trp Met Trp Gln Gly Phe Leu Gly Ser Thr Leu 465 470 475 480 Thr Ser Gly Asp Leu Val Leu Phe Ala Leu Tyr Met Phe Val Ile Ser 485 490 495 Pro Asn Asn Leu Arg Lys Gly Leu Ile Asn His Leu Ile Ser Asp Pro 500 505 510 Thr Gly Ala Thr Met Ile Lys Thr Tyr Leu Lys Val 515 520 4 1848 DNA Adonis palaestina 4 gagagaaaaa gagtgttata ttaatgttac tgtcgcattc ttgcaacaca tattcagact 60 ccattttctt gttttctctt caaaacaaca aactaatgtg acggagtatc tagctatgga 120 actacttggt gttcgcaacc tcatctcttc ttgccctgtc tggacttttg gaacaagaaa 180 ccttagtagt tcaaaactag cttataacat acatcgatat ggttcttctt gtagagtaga 240 ttttcaagtg agggctgatg gtggaagcgg gagtagaact tctgttgctt ataaagaggg 300 ttttgtggac gaggaggatt ttatcaaagc tggtggttct gagcttttgt ttgtccaaat 360 gcagcaaaca aagtctatgg agaaacaggc caagctcgcc gataagttgc caccaatacc 420 tttcggagaa tctgtgatgg acttggttgt aataggttgt ggacctgctg gtctttcact 480 ggctgcagaa gctgctaagc taggcttgaa agttggcctt attggtcctg atcttccttt 540 tacaaataat tatggtgtgt gggaagacga gttcaaagat cttggacttg aacgttgtat 600 cgagcatgct tggaaggaca ccatcgtata tcttgacaat gatgctcctg tccttattgg 660 tcgtgcatat ggacgagtta gccggcattt gctgcatgaa gagttgctga aaaggtgtgt 720 cgagtcaggt gtatcatatc tgaattctaa agtggaaagg atcactgaag ctggtgatgg 780 ccatagtctt gtagtttgtg aaaacgacat ctttatccct tgcaggcttg ctactgttgc 840 atctggagca gcttcaggga aacttttgga gtatgaagta ggtggccctc gtgtttgtgt 900 ccaaactgct tatggtgtgg aggttgaggt ggagaacaat ccatacgatc ccaacttaat 960 ggtatttatg gactacagag actatatgca acagaaatta cagtgctcgg aagaagaata 1020 tccaacattt ctctatgtca tgcccatgtc gccaacaaga cttttttttg aggaaacctg 1080 tttggcctca aaagatgcca tgcctttcga tctactgaag agaaaactaa tgtcacgatt 1140 gaagactctg ggtatccaag ttacaaaaat ttatgaagag gaatggtctt atattcctgt 1200 tgggggttct ttaccaaaca cagagcaaaa gaacctagca tttggtgctg cagcaagcat 1260 ggtgcatcca gcaacaggct attcggttgt acgatcacta tcagaagctc caaaatatgc 1320 ttctgtaatt gcaaagattt tgaagcaaga taactctgca tatgtggttt ctggacaaag 1380 cagtgcagta aacatttcaa tgcaagcatg gagcagtctt tggccaaagg agcgaaaacg 1440 tcaaagagca ttctttcttt tcgggttaga gcttattgtg cagctagata ttgaagcaac 1500 cagaacgttc tttagaacct tcttccgctt gccaacttgg atgtggtggg gtttccttgg 1560 gtcttcacta tcatctttcg atcttgtatt gttttccatg tacatgtttg ttttggcccc 1620 gaacagcatg aggatgtcac ttgtgagaca tttgctttca gatccttctg gtgcagttat 1680 ggttaaagct tacctcgaaa ggtaatctgt tttatgaaac tatagtgtct cattaaataa 1740 atgaggatcc ttcgtatatg tatatgatca tctctatgta tatcctatat tctaatctca 1800 taaagtaatc gaaaattcat tgatagaaaa aaaaaaaaaa aaaaaaaa 1848 5 529 PRT Adonis palaestina 5 Met Glu Leu Leu Gly Val Arg Asn Leu Ile Ser Ser Cys Pro Val Trp 1 5 10 15 Thr Phe Gly Thr Arg Asn Leu Ser Ser Ser Lys Leu Ala Tyr Asn Ile 20 25 30 His Arg Tyr Gly Ser Ser Cys Arg Val Asp Phe Gln Val Arg Ala Asp 35 40 45 Gly Gly Ser Gly Ser Arg Thr Ser Val Ala Tyr Lys Glu Gly Phe Val 50 55 60 Asp Glu Glu Asp Phe Ile Lys Ala Gly Gly Ser Glu Leu Leu Phe Val 65 70 75 80 Gln Met Gln Gln Thr Lys Ser Met Glu Lys Gln Ala Lys Leu Ala Asp 85 90 95 Lys Leu Pro Pro Ile Pro Phe Gly Glu Ser Val Met Asp Leu Val Val 100 105 110 Ile Gly Cys Gly Pro Ala Gly Leu Ser Leu Ala Ala Glu Ala Ala Lys 115 120 125 Leu Gly Leu Lys Val Gly Leu Ile Gly Pro Asp Leu Pro Phe Thr Asn 130 135 140 Asn Tyr Gly Val Trp Glu Asp Glu Phe Lys Asp Leu Gly Leu Glu Arg 145 150 155 160 Cys Ile Glu His Ala Trp Lys Asp Thr Ile Val Tyr Leu Asp Asn Asp 165 170 175 Ala Pro Val Leu Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg His Leu 180 185 190 Leu His Glu Glu Leu Leu Lys Arg Cys Val Glu Ser Gly Val Ser Tyr 195 200 205 Leu Asn Ser Lys Val Glu Arg Ile Thr Glu Ala Gly Asp Gly His Ser 210 215 220 Leu Val Val Cys Glu Asn Asp Ile Phe Ile Pro Cys Arg Leu Ala Thr 225 230 235 240 Val Ala Ser Gly Ala Ala Ser Gly Lys Leu Leu Glu Tyr Glu Val Gly 245 250 255 Gly Pro Arg Val Cys Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val 260 265 270 Glu Asn Asn Pro Tyr Asp Pro Asn Leu Met Val Phe Met Asp Tyr Arg 275 280 285 Asp

Tyr Met Gln Gln Lys Leu Gln Cys Ser Glu Glu Glu Tyr Pro Thr 290 295 300 Phe Leu Tyr Val Met Pro Met Ser Pro Thr Arg Leu Phe Phe Glu Glu 305 310 315 320 Thr Cys Leu Ala Ser Lys Asp Ala Met Pro Phe Asp Leu Leu Lys Arg 325 330 335 Lys Leu Met Ser Arg Leu Lys Thr Leu Gly Ile Gln Val Thr Lys Ile 340 345 350 Tyr Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn 355 360 365 Thr Glu Gln Lys Asn Leu Ala Phe Gly Ala Ala Ala Ser Met Val His 370 375 380 Pro Ala Thr Gly Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro Lys 385 390 395 400 Tyr Ala Ser Val Ile Ala Lys Ile Leu Lys Gln Asp Asn Ser Ala Tyr 405 410 415 Val Val Ser Gly Gln Ser Ser Ala Val Asn Ile Ser Met Gln Ala Trp 420 425 430 Ser Ser Leu Trp Pro Lys Glu Arg Lys Arg Gln Arg Ala Phe Phe Leu 435 440 445 Phe Gly Leu Glu Leu Ile Val Gln Leu Asp Ile Glu Ala Thr Arg Thr 450 455 460 Phe Phe Arg Thr Phe Phe Arg Leu Pro Thr Trp Met Trp Trp Gly Phe 465 470 475 480 Leu Gly Ser Ser Leu Ser Ser Phe Asp Leu Val Leu Phe Ser Met Tyr 485 490 495 Met Phe Val Leu Ala Pro Asn Ser Met Arg Met Ser Leu Val Arg His 500 505 510 Leu Leu Ser Asp Pro Ser Gly Ala Val Met Val Lys Ala Tyr Leu Glu 515 520 525 Arg 6 530 PRT Adonis palaestina 6 Met Glu Leu Leu Gly Val Arg Asn Leu Ile Ser Ser Cys Pro Val Trp 1 5 10 15 Thr Phe Gly Thr Arg Asn Leu Ser Ser Ser Lys Leu Ala Tyr Asn Ile 20 25 30 His Arg Tyr Gly Ser Ser Cys Arg Val Asp Phe Gln Val Arg Ala Asp 35 40 45 Gly Gly Ser Gly Ser Arg Ser Ser Val Ala Tyr Lys Glu Gly Phe Val 50 55 60 Asp Glu Glu Asp Phe Ile Lys Ala Gly Gly Ser Glu Leu Leu Phe Val 65 70 75 80 Gln Met Gln Gln Thr Lys Ser Met Glu Lys Gln Ala Lys Leu Ala Asp 85 90 95 Lys Leu Pro Pro Ile Pro Phe Gly Glu Ser Val Met Asp Leu Val Val 100 105 110 Ile Gly Cys Gly Pro Ala Gly Leu Ser Leu Ala Ala Glu Ala Ala Lys 115 120 125 Leu Gly Leu Lys Val Gly Leu Ile Gly Pro Asp Leu Pro Phe Thr Asn 130 135 140 Asn Tyr Gly Val Trp Glu Asp Glu Phe Lys Asp Leu Gly Leu Glu Arg 145 150 155 160 Cys Ile Glu His Ala Trp Lys Asp Thr Ile Val Tyr Leu Asp Asn Asp 165 170 175 Ala Pro Val Leu Ile Gly Arg Ala Tyr Gly Arg Val Ser Arg His Leu 180 185 190 Leu His Glu Glu Leu Leu Lys Arg Cys Val Glu Ser Gly Val Ser Tyr 195 200 205 Leu Asp Ser Lys Val Glu Arg Ile Thr Glu Ala Gly Asp Gly His Ser 210 215 220 Leu Val Val Cys Glu Asn Glu Ile Phe Ile Pro Cys Arg Leu Ala Thr 225 230 235 240 Val Ala

Ser Gly Ala Ala Ser Gly Lys Leu Leu Glu Tyr Glu Val Gly 245 250 255 Gly Pro Arg Val Cys Val Gln Thr Ala Tyr Gly Val Glu Val Glu Val 260 265 270 Glu Asn Asn Pro Tyr Asp Pro Asn Leu Met Val Phe Met Asp Tyr Arg 275 280 285 Asp Tyr Met Gln Gln Lys Leu Gln Cys Ser Glu Glu Glu Tyr Pro Thr 290 295 300 Phe Leu Tyr Val Met Pro Met Ser Pro Thr Arg Leu Phe Phe Glu Glu 305 310 315 320 Thr Cys Leu Ala Ser Lys Asp Ala Met Pro Phe Asp Leu Leu Lys Arg 325 330 335 Lys Leu Met Ser Arg Leu Lys Thr Leu Gly Ile Gln Val Thr Lys Val 340 345 350 Tyr Glu Glu Glu Trp Ser Tyr Ile Pro Val Gly Gly Ser Leu Pro Asn 355 360 365 Thr Glu Gln Lys Asn Leu Ala Phe Gly Ala Ala Ala Ser Met Val His 370 375 380 Pro Ala Thr Gly Tyr Ser Val Val Arg Ser Leu Ser Glu Ala Pro Lys 385 390 395 400 Tyr Ala Ser Val Ile Ala Lys Ile Leu Lys Gln Asp Asn Ser Ala Tyr 405 410 415 Val Val Ser Gly Gln Ser Ser Ala Val Asn Ile Ser Met Gln Ala Trp 420 425 430 Ser Ser Leu Trp Pro Lys Glu Arg Lys Arg Gln Arg Ala Phe Phe Thr 435 440 445 Leu Phe Gly Leu Glu Leu Ile Val Gln Leu Asp Ile Glu Ala Thr Arg 450 455 460 Thr Phe Phe Arg Thr Phe Phe Arg Leu Pro Thr Trp Met Trp Trp Gly 465 470 475 480 Phe Leu Gly Ser Ser Leu Ser Ser Phe Asp Leu Val Leu Phe Ser Met 485 490 495 Tyr Met Phe Val Leu Ala Pro Asn Ser Met Arg Met Ser Leu Val Arg 500 505 510 His Leu Leu Ser Asp Pro Ser Gly Ala Val Met Val Arg Ala Tyr Leu 515 520 525 Glu Arg 530

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